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Immunology (plasmodium falciparum) Poor Antibody recognition of MSP-1 Product observed

Infec. & Immun, August 1997; 65:3024-3031

Infec. & Immun., 1996; 64:253

Nature 1984; 311:379-82

Infec. & Immun., September 1996; 64(9):3614-3619

Infec. & Immun, July 1996; 64(7):2716-2723

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L4 ANSWER 1 OF 17 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1997:392505 BIOSIS
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TI Characterization of **human** T- and B-cell **epitopes** in the **C terminus** of **Plasmodium falciparum** merozoite **surface protein 1** : Evidence of poor T-cell recognition of polypeptides with numerous disulfide bonds.
AU Egan, Andrea; Waterfall, Martin; Pinder, Margaret; Holder, Anthony; Riley, Eleanor (1)
CS (1) Inst. Cell Anim. Population Biol., Div. Biological Sci., Univ. Edinburgh, Ashworth Lab., West Mains Rd., Edinburgh EH9 3JT UK
SO Infection and Immunity, (1997) Vol. 65, No. 8, pp. 3024-3031. ISSN: 0019-9567.
DT Article
LA English
AB We have investigated the relationship between cellular and humoral immune responses to defined **epitopes** of the **C terminus** of merozoite **surface protein 1** (**MSP-1**) of the **human** malaria parasite, **Plasmodizium falciparum**, in immune blood donors. Sera from almost all donors contained antibodies to the 33-kDa processing product of the MAD20 allele of **MSP-1** ( **MSP-1**-33), but these antibodies did not cross-react with the equivalent sequence of the Wellcome allele. In contrast, T-cell responses to **MSP-1**-33 are directed towards **epitopes** that are conserved between the two allelic families. Only 50% of adult blood donors possessed antibodies which recognized the **19-kDa** processing product of **MSP-1** ( **MSP-1-19**). These antibodies predominantly recognized conserved **epitopes** involving both of the constituent epidermal growth factor-like domains of **MSP-1-19**. T-cell responses were found in only 26% (for recombinant proteins) or 44% (for synthetic peptides) of donors and were directed mainly at dimorphic sequences of the protein. There was no obvious association, at an individual level, between the presence of antibodies and the detection of T-cell proliferative or gamma interferon responses, suggesting that the T cells identified in this manner are not providing significant levels of help to B cells. T-cell responses to **reduced** recombinant proteins and linear peptides were more prevalent than responses to disulfide-bonded proteins, suggesting that the complex disulfide-bonded structure of native **MSP-1-19** , may inhibit antigen processing or presentation.

L4 ANSWER 2 OF 17 CABA COPYRIGHT 1999 CABI
AN 1999:92820 CABA
DN 990805283
TI Antibodies against **Plasmodium falciparum** vaccine candidates in infants in an area of intense and perennial transmission: relationships with clinical malaria and with entomological inoculation rates
AU Kitua, A. Y.; Urassa, H.; Wechsler, M.; Smith, T.; Vounatsou, P.; Weiss, N. A.; Alonso, P. L.; Tanner, M.
CS Ifakara Centre, PO Box 53, Ifakara, Tanzania.
SO Parasite Immunology, (1999) Vol. 21, No. 6, pp. 307-317. 54 ref. ISSN: 0141-9838
DT Journal
LA English
AB In 1993-1994, serum immunoglobulin (Ig)G1, IgG3 and total IgG were

Immunology (Plasmodium falciparum) Poor Antibody Recognition of MSP-1 Product Observed
Vaccine Weekly Sep 8, 1997 p. N/A
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The complex disulfide-bonded structure of native MSP-1(19) might inhibit antigen processing.

Andrea Egan, University of Edinburgh, Edinburgh, United Kingdom, and colleagues studied the relationship between cellular and humoral immune responses to defined epitopes of the C terminus of merozoite surface protein 1 (MSP-1) of Plasmodium falciparum in immune blood donors ("Characterization of Human T- and B-Cell Epitopes in the C Terminus of Plasmodium falciparum Merozoite Surface Protein 1: Evidence for Poor T-Cell Recognition of Polypeptides with Numerous Disulfide Bonds," Infection and Immunity, August 1997;65:3024-3031).

MSP-1 has been the focus of attention in the pursuit of a subunit vaccine to extend immunity to P. falciparum malaria. Studies of the naturally immunogenic protein have demonstrated that immunization of nonhuman primates with native or recombinant MSP-1 confers varying degrees of resistance to challenge infection with P. falciparum (Chang et al., Infect Immun, 1996;64:253; Hall et al., Nature, 1984;311:379-382; and others).

"Sera from almost all donors contained antibodies to the 33-kDa processing product of the MAD20 allele of MSP-1 (MSP-1(33)), but these antibodies did not cross-react with the equivalent sequence of the Wellcome allele," wrote Egan et al. "In contrast, T-cell responses to MSP-1(33) are directed towards epitopes that are conserved between the two allelic families. Only 50 percent of adult blood donors possessed antibodies which recognized the 19-kDa processing product of MSP-1 (MSP-1(19))."

These antibodies primarily recognized conserved epitopes that involved both of the constituent epidermal growth factor-like domains of MSP-1(19), the authors said.

They found T-cell responses in only 26 percent (for recombinant proteins) or 44 percent (for synthetic peptides) of donors, and the responses were directed mostly at dimorphic sequences of the protein. They reported no obvious association, at the individual level, between the presence of antibodies and the detection of T-cell proliferative or gamma interferon responses. Egan et al. said their findings suggest that the T cells identified in this way are not furnishing substantial levels of help to the B cells.

"T-cell responses to reduced recombinant proteins and linear peptides were more prevalent than responses to disulfide-bonded proteins, suggesting that the complex disulfide-bonded structure of native MSP-1(19) may inhibit antigen processing or presentation," Egan et al. wrote.

The researchers noted that their earlier studies showed that both antibody and T-cell responses to MSP-1(42) were associated with resistance to clinical malaria in African children (Riley et al., Parasite Immunol, 1992;14:321-338).

Additional study of the antibody response to MSP-1(42) suggested that protective epitopes lie within both MSP-1(19) (Egan et al., Infect Immun, 1995;63:456-466) and MSP-1(33) (Egan. 1995. Ph.D. thesis. University of Edinburgh, Edinburgh, United Kingdom), the researchers said. Additionally, they indicated that the low prevalence of T-cell responses to MSP-1(19) suggests that the protective T-cell responses they detected in response to MSP-1(42) actually were induced by epitopes within MSP-1(33).

"Taken together, these data suggest that the dimorphic 33-kDa fragment

of MSP-1 should be further evaluated as a potential vaccine antigen," Egan et al. wrote.

This study received financial support from The Wellcome Trust and the UK Medical Research Council.

The corresponding author for this study is Eleanor Riley, Institute of Cell, Animal and Population Biology, Division of Biological Sciences, University of Edinburgh, Ashworth Laboratories, West Mains Road, Edinburgh EH9 3JT, United Kingdom.

- by Cathy Clark


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# Major surface antigen gene of a human malaria parasite cloned and expressed in bacteria

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**The late blood stages of the human malaria parasite, *Plasmodium falciparum*, carry a major surface antigen, p190, of molecular weight ($M_r$) 190,000 (ref. 1). This antigenically variable protein[1–3] is actively processed, first as the parasite matures and again when it is released into the blood stream and invades a new erythrocyte to initiate a cycle of growth[1]. It elicits a strong immune response in man; all tested adult sera from endemic areas have antibodies against this protein[1]. Our evidence indicates that purified p190 can alter the course of parasitaemia in monkeys with falciparum malaria. We have also succeeded in cloning part of the gene for p190 and expressing it in *Escherichia coli*. To this end we have developed a new technique, antibody select, which greatly simplifies final identification of expressing clones.**

The protein p190 was first defined[2] and later purified[1] by monoclonal antibodies. It begins to be made in schizogony as a 190,000 $M_r$ protein. Processing creates smaller peptides, notably major species of approximately 160,000, 125,000, 106,000 and 82,000 $M_r$ (ref. 1 and R.H., unpublished). These and other features strongly suggest that p190 is related to the 195,000 $M_r$ protein studied by Holder and Freeman[4], and to similar proteins in rodent[5–7] and simian[8,9] parasites. One part of p190, defined by monoclonal antibody 2.2, is present in all isolates of *P. falciparum* tested; another part, defined by antibody 7.3, is present in p190 purified from the Thai isolate K1 (ref. 10) but absent from some other isolates[2,3]. Genetically pure lines from a single Thai patient vary in this part of the protein[11]. The antigenically variable part of the protein is cleaved off during release and re-invasion, whilst the constant part survives into the next cycle[1].

Preliminary studies suggest that p190 can protect monkeys against falciparum malaria. Immunization with the affinity-purified native protein modifies the course of infection by the parasite. We treated four control monkeys (Fig. 1) with Freund's complete adjuvant for the first immunization only and with incomplete adjuvant for subsequent immunizations before challenge with the parasite. All show a rapid rise in parasitaemia to the level (20%) at which drug treatment is given to prevent death of the animals. By contrast, two of the three immunized monkeys after an initial rise, rapidly lose detectable parasites and recover. All the immunized monkeys develop antibodies against p190 and show an anamnistic response after challenge (Table 1). Interestingly, the variable part of the p190 protein used in this experiment (purified from isolate K1), was different from its homologue in the challenge parasite (Palo Alto strain)[2,3]. This suggests that the constant part of p190 may have protective value.

Much could be learned about this protein from cloning its gene. In addition, clones expressing the gene in bacteria would be a ready source of a protein which is a strong candidate for a subunit vaccine against falciparum malaria. Recently, DNA sequences encoding antigens from both *P. knowlesi*[12] and *P. falciparum*[13] have been cloned. These studies showed that clones

**Table 1 Antibody response to p190 immunization**

| Monkey | Inoculated with: | Antibodies against p190 (reciprocal titres) *a* | *b* | *c* |
|---|---|---|---|---|
| Sven | FCA | <100 | <100 | 100 |
| Uli | FCA | <100 | <100 | 100 |
| Dora | FCA | <100 | <100 | 900 |
| Ronnie | FCA | <100 | 100 | 100 |
| Laura | p190+FCA | <100 | 24,300 | >72,900 |
| Anita | p190+FCA | 100 | 8,100 | >72,900 |
| Hugo | p190+FCA | <100 | 24,300 | >72,900 |

Anti-p190 antibodies assayed by ELISA in the treated monkeys. *a*, Before immunization; *b*, after immunization; *c*, 30 days after challenge. FCA, Freund's complete adjuvant. Microtitre plates (Nunc) were coated with affinity-purified p190: 50 µl containing ~100 ng were added to each well and incubated (room temperature; overnight), washed thoroughly (×5) with tap water. Remaining sites were blocked with 100 µl of bovine serum albumin (BSA) solution (3% in phosphate-buffered saline, PBS) for 30 min (room temperature). Threefold dilutions (50 µl) of monkey sera (in 1% BSA in PBS) were incubated (room temperature; 6 h) in each well; the plate was washed (tap water) before adding urease-conjugated rabbit anti-Saimiri IgG antiserum (50 µl of an appropriate dilution in 1% BSA (as above), overnight at room temperature and washed again (×4 in tap water ×2 in distilled water). 150 µl of urease substrate (urea, 0.1%; 0.008% bromocresol purple; 0.2 mM EDTA; *p*H 4.8) was added to each well and incubated (37 °C, 45 min). A positive reaction registers as a change from purple to yellow[27].

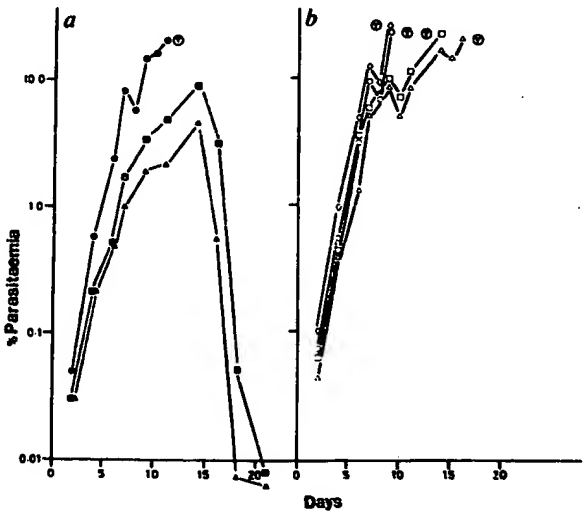


**Fig. 1** Effect of p190 on the course of parasitaemia in Saimiri monkeys. *a*, Immunized monkeys. Affinity-purified p190 was injected into three monkeys with Freund's complete adjuvant—Anita (●), Hugo (■) and Laura (▲). Each received subcutaneously 50 µg at day −52, 50 µg at day −39 and 50 µg at day −19. *b*, Controls (open symbols) received adjuvant alone. After immunization, all the monkeys were challenged intravenously with ~$2 \times 10^7$ parasitized erythrocytes (*P. falciparum*, Palo Alto strain) from a single donor monkey. ⓓ indicates drug treatment. Parasitaemias were estimated by Giemsa-stained smears.

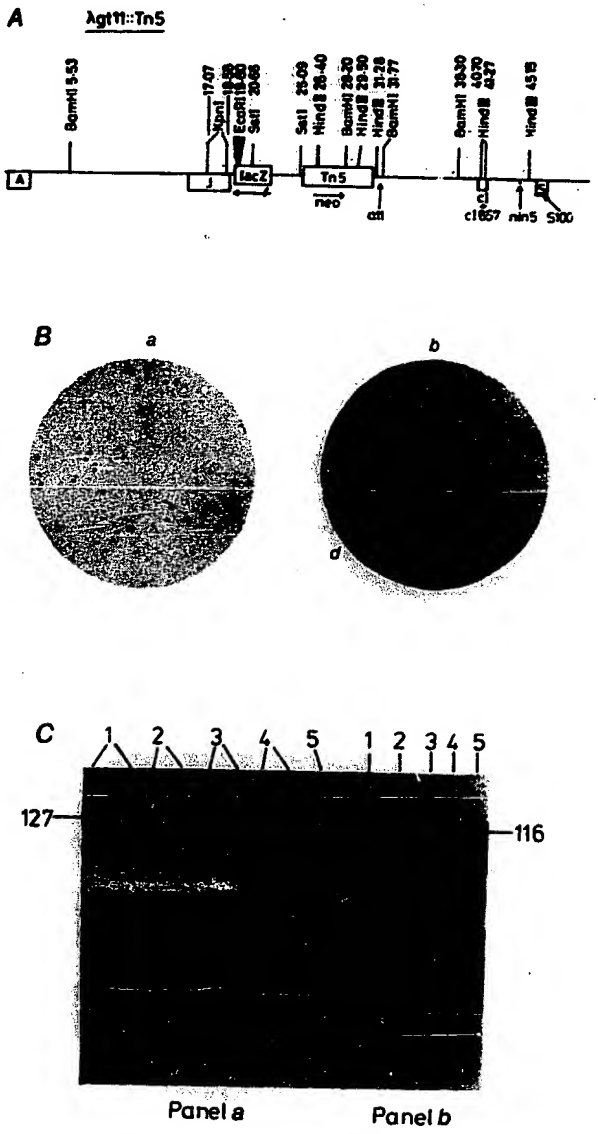


**Fig. 2** Isolation of cDNA clones containing p190 sequences. *A*, Cloning vector. Phage λ*gt11*::Tn*5* was isolated following transposition of Tn*5* to λ*gt11*[14]. The cDNA is inserted at the unique *Eco*RI site in *lacZ*, positions of important restriction sites are indicated in kb. The phage is temperature-inducible ($cI_{857}$) and carries the *nin5* deletion and an amber mutation (S100) preventing lysis in the absence of a suppressor. The presence of Tn*5* greatly simplifies isolation of lysogens, as these derivatives are resistant to kanamycin. *B*, Colony screening of the λ*gt11*::Tn*5* cDNA expression library by ELISA. *a*, Positive colonies from the primary screen transferred to nitrocellulose and probed again with rabbit anti-p190 serum. *b*, p190 negative colonies from the primary screen probed with anti-p190 serum. *c*, Positive colonies from the primary screen probed with non-immune rabbit serum. *d*, p190-negative colonies from the primary screen probed with anti-β-galactosidase serum. *C*, Western blots of total protein from four p190 lysogens probed with anti-p190 serum (panel *a*, lanes 1–4, each clone run in duplicate) and anti-β-galactosidase serum (panel *b*, lanes 1–4). Lane 5 in each panel shows a control lysogen carrying the vector with no cDNA insert.

**Methods:** The λ*gt11*::Tn*5* cDNA library in BTA282, Δ$lac_{U169}$Δ*lon araD*139 $str^R$ *thi hfl* A150 (chr::Tn*10*) *hsd*$R^-M^+$ (a derivative of RY1083, see ref. 14) was grown overnight at 30 °C on L-agar with 1555 μg $ml^{-1}$ tetracycline and 40 μg $ml^{-1}$ kanamycin. Colonies were replicated onto nitrocellulose and incubated 2 h at 30 °C. Phage were induced by incubation at 42 °C for 90 min. Colonies were lysed by a modification of the method of Stanley[28]. Nitrocellulose disks were placed on a pad soaked in 5% SDS and heated in a 100 °C oven for 15 min. They were immersed (1 h, 20 °C) in 5% ovalbumin in 10 mM Tris-Cl, *p*H 8.0, 150 mM NaCl (5% OA/TS) containing 0.01% $NaN_3$, 0.2 mg $ml^{-1}$ henylmethyisulphonyl fluoride (PMSF) and 10 μg $ml^{-1}$ DNase I, washed twice in TS and electroeluted (1 h, 50 V) to remove SDS. The filters were further blocked in 5% OA/TS (15 min, 20 °C) and incubated overnight in rabbit anti-p190 serum diluted 1:150 in 5% OA/TS (5 ml). They were washed 5× in TS and incubated in 1:1,000 dilution of peroxidase-conjugated goat anti-rabbit IgG in 5% OA/TS (4 h, 20 °C). After a further five washes in TS, the ELISA reaction was developed[29]. The anti-p190 serum was raised in a New Zealand White rabbit against affinity-purified p190 protein[1]. It was extensively preadsorbed on induced λ*gt11*::Tn*5* lysogens of BTA282 to remove nonspecific anti-*E. coli* antibodies. Total proteins from lysogens for gel analysis were obtained as follows: Young L-broth cultures of lysogens (in BTA282), containing 15 μg $ml^{-1}$ tetracycline and 40 μg $ml^{-1}$ kanamycin, were induced (90 min, 42 °C), centrifuged (1,500*g*, 10 min) and the bacteria (~$2\times10^9$) resuspended in 60 μl of 10% SDS, 0.1 mg $ml^{-1}$ PMSF (15 min, 20 °C) and boiled (2 min). 180 μl of Laemmli[30] sample buffer containing 7% 2-mercaptoethanol was added and after further boiling, the samples were electrophoresed on 7.5% SDS-polyacrylamide gels and Western blotted essentially as described previously[29,31]. The filters were probed with antibody as above. Markers used to calibrate the gels were ferritin (220,000 $M_r$), phosphorylase *b* (94,000 $M_r$), bovine serum albumin (69,000), catalase (60,000), ovalbumin (46,000), lactate dehydrogenase (36,000) and carbonic anhydrase (30,000).

can be detected using antibody probes to screen bacteria expressing parts of the parasite protein. We report here isolation of a p190 cDNA clone detected by a similar strategy. It includes the 3′ terminus of the messenger RNA and the sequence encoding the final 70 aminoacids at the C-terminus of the protein.

We have made a useful derivative of phage λ*gt11* to serve as the cloning vector for cDNA from cultured *P. falciparum* blood stages. Like its parent,[14,15] our derivative (λ*gt11*::Tn*5*) facilitates expression of cloned cDNA in *E. coli*. The transposon Tn*5* confers kanamycin resistance on lysogens, which can thus be very easily selected (M.G., N. Bone and J.S., unpublished results). The cDNA was made from total poly(A)$^+$ RNA of blood stage *P. falciparum* isolate KI, ligated to *Eco*RI linkers and cloned[16] into the unique *Eco*RI site of the vector. The cDNA is thereby inserted int the *lacZ* gene, encoding β-galactosidase (Fig. 2*A*). In the correct frame and orientation at this site, a cDNA molecule is expressed as a hybrid protein comprising most of the β-galactosidase molecule fused to a *P. falciparum* polypeptide (the *Eco*RI site is 153 base pairs (bp) from the C-terminus of the gene)[14,17].

Recombinant phages (~7,000) were screened for synthesis of p190 peptides. Small colonies lysogenic for the phages were transferred to nitrocellulose, induced to stimulate fusion protein synthesis and probed (see Fig. 2) with a polyclonal rabbit antiserum raised against affinity-purified p190[1]. Positive candidate clones were detected by enzyme-linked immunosorbent assay (ELISA) with horseradish peroxidase-conjugated goat anti-rabbit IgG antiserum (Fig. 2*B*). Four apparently identical clones were obtained from this screening. They each encode a hybrid protein (Fig. 2*C*) significantly larger (127,000 $M_r$) than native β-galactosidase[15]. In Western blots (Fig. 2*C*) they can be seen to have epitopes recognized by both the anti-β-galactosidase (right panel) and the anti-p190 (left panel) probes. By contrast, control clones synthesizing native enzyme only respond to the anti-β-galactosidase probe.

To confirm that our clones encode a p190 sequence, we have developed a rapid and simple technique which we call antibody select. This method eliminates false positive clones which could register in the initial screening if, for example, the anti-p190 probe contained antibodies against other *P. falciparum* pro-

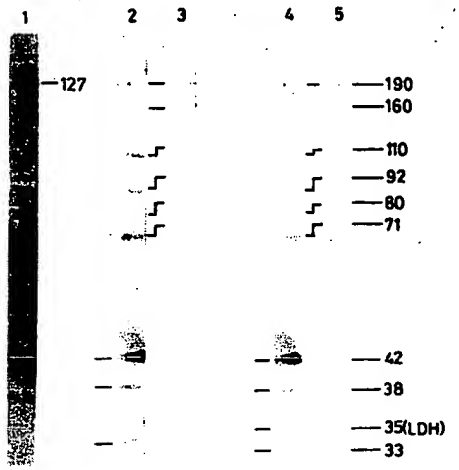


Fig. 3 Confirmation of a λp190 clone by antibody select. Lane 1, p190 hybrid protein used in antibody select (see lanes 3, 5). The protein isolated from an induced lysogen (see below) and run on an SDS-polyacrylamide gel was stained with Coomassie blue. The major band is at 127,000 $M_r$ (see Fig. 2C). Lanes 2–5, Western blots of total KI parasite proteins using the following probes: lane 2, whole anti-p190 serum; lane 3, immunoglobulins affinity-purified from the whole serum by the hybrid protein; lane 4, whole serum with added rabbit antibody against parasite lactate dehydrogenase (LDH); lane 5, immunoglobulins affinity-purified from the mixed sera of lane 4 by the hybrid protein.

**Methods:** p190 hybrid protein for antibody select was purified as follows: Approximately $10^{11}$ lysogens (in BTA282) harbouring p190-1 were grown up and collected as described in Fig. 2 legend. The bacterial pellet was resuspended in 4 ml cold 10 mM Tris-Cl, *p*H 8.0, 100 mM NaCl, 1 mM EDTA (TNE) and left on ice 5 min. 4 ml of 50% sucrose in TNE were added, followed 10 min later by 8 ml of 4 mg $ml^{-1}$ lysozyme. After a further 15 min on ice, 4 ml of 0.25 M EDTA, *p*H 8.0 and 2 ml of 10% Nonidet P-40 were added. The suspension was freeze-thawed and the DNA sheared by 5× extrusion through a 19-gauge needle. The extract was then spun at 13,000*g*, 4 °C, 30 min and the pellet suspended in 15 ml of 8 M urea. After a further spin (13,000*g*, 4 °C, 20 min), the supernatant, which contained purified fusion protein, was retained. For antibody select, 4 ml of this supernatant was incubated with 5 $cm^2$ of nitrocellulose (2 h, 20 °C). After washing and blocking the filter as described for Fig. 2, it was incubated with 5 ml of undiluted rabbit anti-p190 serum plus 5 ml 5% OA/TS overnight at 20 °C. After five washes in TS, specifically bound antibodies were eluted by incubation with 2 ml of 0.1 M glycine-Cl, *p*H 2.6, 0.15 M NaCl (15 min, 20 °C). The eluted material was neutralized with 2 ml 1 M Tris-Cl *p*H 8.0 and 16 ml 5% OA/TS plus 0.01% $NaN_3$ were added. This solution was used to probe Western blots (lanes 3, 5). Total KI proteins were prepared from parasite pellets (~$2\times10^9$ mixed stage parasites) after saponin lysis of erythrocytes[1]. The pellets were boiled in 500 μl of Laemmli sample buffer and after spinning on a microfuge (5 min) 80 μl of the supernatant were run on a 7.5% SDS-polyacrylamide gel.

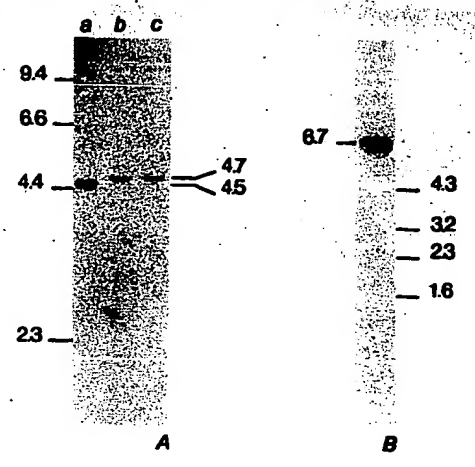


Fig. 4 Parasite sequences hybridizing to the λp190-1 cDNA insert. Panel *A*, Southern blots of *Eco*RI cleaved genomic DNA from different isolates of *P. falciparum*. Lane *a*, isolate KI (Thailand)[10]; lane *b*, isolate MAD20 (Papua, New Guinea)[3]; lane *c*, Tak9 clone 96 (Thailand)[21]. Markers are *Hin*dIII fragments of $\lambda cI_{857}$ (kb). Panel *B*, Northern blot of total poly(A)$^+$ RNA from isolate KI. Markers are *E. coli* 16S and 23S RNA and *P. falciparum* '18S' and '28S' RNA[20] (kb).

**Methods:** For Southern blots 6 μg of genomic DNA were cleaved with 10 U of *Eco*RI (overnight, 37 °C) and electrophoresed on a 0.7% agarose gel. For Northern blots 3 μg of poly(A)$^+$ RNA were run on a 0.8% agarose gel in 2.2 M formaldehyde[20]. Both DNA and RNA were transferred to nitrocellulose in 1 M $NH_4Ac$, 0.02 M NaOH (1 h). The probe was prepared by digesting λp190-1 DNA with *Kpn*I and *Sst*I to yield a 2.4 kb fragment which includes the 300 bp cDNA insert (see Fig. 2A). This was separated from other fragments on a preparative 0.6% agarose gel and after purification, nick-translated with [$\alpha^{32}$P]dCTP to a specific activity of $1.7\times10^7$ c.p.m. $\mu g^{-1}$. After hybridization, filters were washed to a stringency of 0.1 ×SSC at 37 °C.

tein(s). Such proteins can be minor contaminants in the p190 preparation used to raise the polyclonal rabbit antiserum.

The hybrid protein of a true p190 clone should be able to bind specific anti-p190 immunoglobulin molecules, permitting them to be affinity purified from the original anti-p190 probe. By contrast, the hybrid proteins of false clones would select immunoglobulin molecules against the contaminating protein.

Hybrid protein from a candidate p190 clone (λp190-1) was bound to nitrocellulose and used to affinity-purify antibodies from the original probe (Fig. 3). The selected antibodies, when eluted and used t probe Western blots f total parasite proteins, prove t bind t p190 and its processed products (160,000, 110,000, 92,000, 80,000 and 71,000 $M_r$). Moreover, the selection is quite specific. When antibodies against another parasite protein (lactate dehydrogenase, $M_r$ 35,000) were added to the anti-p190 probe, these were not selected (compare lanes 4 and 5), nor were antibodies already present in the probe against 42,000, 38,000 and 33,000 $M_r$ contaminating proteins (compare lanes 2 and 3).

Antibody select is a rapid and convenient way to confirm or identify any foreign gene sequences expressed in bacteria. It provides an alternative to both hybrid selection of mRNA[18], which requires a large amount of mRNA from the parent organism, and immunization of mice with the fusion protein, followed by immuno-identification of the parent protein in extracts[13,19]. This latter approach is slow and we find that fusion proteins do not always elicit a strong immune response. Antibody select has the added advantage that the antibody probe used in the initial identification of the clones provides the immunoglobulins for the confirmation step. It also provides a way to purify from complex sera antibodies against defined regions of a single antigen molecule.

The clones and their hybrid protein are stable during growth in *E. coli*. Their inserts, which can all be excised with *Eco*RI, hybridize strongly to a total cDNA probe (made from blood-stage parasite poly(A)$^+$ RNA) and give an identical fragment of approximately 300 bp (data not shown). We have therefore studied one clone, representing a small fraction of the protein coding sequence in detail. We can detect the p190 message in total poly(A)$^+$ RNA extracted from mixed blood stages of the parasite. In Northern blots of denaturing gels[20] it has an apparent length of 6.7 kilobases (kb) (Fig. 4*b*), which agrees with the estimate that a coding sequence of at least 5 kb would be required f r a 190,000 $M_r$ protein.

The gene can be detected in total gen mic DNA. When cut with *Eco*RI, it reveals a single maj r band which hybridizes strongly t the probe (Fig. 4*a*). Interestingly, when we compare DNA from three different isolates, we find a small but significant difference between KI (4.5 kb) and tw others, MAD20 and the

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                10          20          30          40          50
GAATTCCAGTTTAATTTATTAAGAGCAAAATTAAATGATGTATGTGCTAATGATTAT
GluPheGlnPheAsnLeuLeuArgAlaLysLeuAsnAspValCysAlaAsnAspTyr

      60          70          80          90         100         110
TGTCAAATACCTTTCAATCTTAAAATTCGTGCAAATGAATTAGACGTACTTAAAAAACTT
CysGlnIleProPheAsnLeuLysIleArgAlaAsnGluLeuAspValLeuLysLysLeu

     120         130         140         150         160         170
GTGTTCGGATATAGAAAACCATTAGACAATATTAAAGATAATGTACGAAAAATGGAGATT
ValPheGlyTyrArgLysProLeuAspAsnIleLysAspAsnValGlyLysMetGluIle

     180         190         200         210         220         230
ACATTAAAAAAAATAAAAAACCCATAGAAAATATAAATTGAATTAATTTGAAGAAAGTG
ThrLeuLysLysIleLysLysProIleGluAsnIleAsn***

     240         250         260         270         280         290
TAAGAAAACAATTTGATTAAAAATTAAGAATTGCCAACTAAAAAAAAAAAAAAAAAAAAA

AAAAAGGAATTC
```

**Fig. 5** Nucleotide sequence of λp190-1 cDNA. Sequence determination was carried out by the chain termination method[32] after subcloning *Eco*RI fragments of λp190-1 cDNA into M13mp9 (ref. 33) in both orientations. The putative polyadenylation signal is underlined. The reading frame is shown in phase with β-galactosidase. The two *Eco*RI linkers are indicated by a line above the sequence.

genetically pure clone Tak9/96 (4.7 kb). KI and Tak9/96 are from Thailand[10,21], whilst MAD20 is from Papua, New Guinea[3]. This result may be particularly important as MAD20 and Tak9/96 differ from KI with respect to the antigenic properties of p190[2,3].

We have estimated the frequency of the cloned p190 sequence in the *P. falciparum* DNA from the intensity of the 4.5 kb band in Southern blots of total genomic digests. Standard copy-number analysis[22,23] using the λp190-1 clone suggests that the number of copies of the p190 sequence per parasite genome is between one and five (data not shown). The part of the gene in p190-1 does not contain tandem repeats (see below and Fig. 5) as has recently been reported in coding sequences for *P. falciparum* S-antigens[13] and *P. knowlesi* circumsporozoite protein[12].

Sequencing studies (Fig. 5) in this laboratory on the p190-1 insert reveals a single open reading frame encoding 70 amino acids in phase with β-galactosidase, terminating with a UGA codon followed downstream by two other stop codons. The clone has a run of 26 A residues at its 3′ terminus. In addition, the sequence ATTAAA starts 24 bases upstream from the first terminal adenine, the expected position for a polyadenylation signal. This function has recently been attributed to the above sequence in several different systems[24–26]. We therefore suggest that the cloned sequence encodes the 3′-terminus of the p190 mRNA and the C-terminus of the polypeptide.

Our conclusion affects the interpretation of the mechanism by which p190 is processed from a primary product of 190,000 $M_r$ to apparently smaller products. Examination of the parasite proteins reacting to the antibodies selected by the p190 clone reveals that in addition to p190, large product bands are also found (Fig. 3, lanes 3, 5). This strongly suggests that epitopes in the C-terminus are not removed by processing. The simplest interpretation of our result is that processing removes the N-terminus of the p190 polypeptide.

We have been unable to detect in the polypeptide encoded in p190-1 epitopes defined by monoclonal antibodies, including 2.2 and 7.3. This may simply reflect the fact that p190-1 contains less than a tenth of the total coding sequence. An alternative explanation is that the C-terminus of p190 cannot elicit an immune response because it is sequestered inside the parasite, as the monoclonal antibodies were obtained after immunization Hwith a whole parasite preparation[2].

The present report describes a cloned gene for a *P. falciparum* protein known to be both strongly immunogenic in man and a candidate for a subunit vaccine against malaria. We now have clones with larger p190 sequences which will test wheth HHlike the native protein (and its homologue in *P. yoeli* p190 synthesized in bacteria can elicit a protective respo against blood stages of the parasite.

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# Human antisera detect a *Plasmodium falciparum* genomic clone encoding a nonapeptide repeat

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***Plasmodium falciparum*** **causes malaria infectious in its huma host. Its wide distribution in tropical countries is a major worl health problem. Before a vaccine can be produced, the identifica tion and characterization of parasite antigens is necessary. Thi can be achieved by the cloning and subsequent analysis of gene coding for parasite antigens[1–4]. Recently established cDNA bank allow the expression of cDNA derived from the simian parasit *Plasmodium knowlesi*[5] and *P. falciparum*[6,7] in *Escherichia col* Recombinants encoding parasite antigens have been identified b immunodetection in both banks. Two of them contain repetitiv units of 11 (ref. 7) or 12 (ref. 5) amino acids. We describe her the construction of an expression bank made directly from ran domly generated fragments of *P. falciparum* genomic DNA. W detect several clones which react strongly with human Africa immune sera. One clone expresses an antigenic determinant com posed of occasionally degenerated repeats of a peptide nonamer**